

**APPENDIX A - SPECIFICATION/CLAIM AMENDMENTS
INCLUDING NOTATIONS TO INDICATE CHANGES MADE**

Serial No.: 09/814,252

Docket No.: 180.0003 0103

Amendments to the following are indicated by underlining what has been added and bracketing what has been deleted. Additionally, all amendments have been bolded.

In the Specification

The paragraph beginning at page 1 (immediately after the "Statement of Related Applications" heading) has been replaced with the following paragraph.

This application is a divisional of U.S. Patent [Application Serial No. 09/407,818] **No. 6,242,223, issued 05 June 2001**, [filed September 28, 1999,] entitled "Primers for Use in Detecting Beta-Lactamases," which claims the benefit of U.S. Patent Application Serial No. 60/102,181, filed September 28, 1998, entitled "Primers for Use in Detecting Beta-Lactamases," and U.S. Patent Application Serial No. 60/121,765, filed February 26, 1999, also entitled "Primers for Use in Detecting Beta-Lactamases." All three applications are incorporated herein by reference.

In the Claims

For convenience, all pending claims are shown below.

1. (AMENDED) A primer selected from the group of:
5' - CTT GGT CTG ACA GTT ACC - 3' (SEQ ID NO:3);
5' - TGT CGC CCT TAT TCC - 3' (SEQ ID NO:4);
5' - TCG GGG AAA TGT GCG - 3' (SEQ ID NO:5); and **full-length** complements thereof.
2. (AMENDED) A primer selected from the group of:
5' - ATC GTC CAC CAT CCA CTG CA - 3' (SEQ ID NO:6);
5' - GGG AAA CGG AAC TGA ATG AG - 3' (SEQ ID NO:7);
5' - TAG TGG ATC TTT CGC TCC AG - 3' (SEQ ID NO:8);
5' - GCT CTG CTT TGT TAT TC - 3' (SEQ ID NO:9);
5' - CAC TCA AGG ATG TAT TGT G - 3' (SEQ ID NO:10); and **full-length** complements thereof.

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4. (AMENDED) A primer selected from the group of:
5' - CTG GCA ACC ACA ATG GAC TCC G - 3' (SEQ ID NO:18);
5' - GCC AGT TCA GCA TCT CCC AGC C - 3' (SEQ ID NO:19); and full-length
complements thereof.
5. (AMENDED) A primer selected from the group of:
5' - CGT GAC CAA CAA CGC CCA GC - 3' (SEQ ID NO:20);
5' - CCA GAT AGC GAA TCA GAT CGC - 3' (SEQ ID NO:21); and full-length
complements thereof.
7. (AMENDED) A primer selected from the group of:
5' - GGC ATT GGG ATA GTT GCG GTT G - 3' (SEQ ID NO:24);
5' - TTA CTA CAA GGT CGG CGA CAT GAC C - 3' (SEQ ID NO:25); and full-length
complements thereof.
8. (AMENDED) A primer selected from the group of:
5' - GGA TCA CAC TAT TAC ATC TCG C - 3' (SEQ ID NO:26);
5' - CGT ATG GTT GAG TTT GAG TGG C - 3' (SEQ ID NO:27); and full-length
complements thereof.
9. (AMENDED) A primer selected from the group of:
5' - GCG ACC TGG TTA ACT ACA ATC CC - 3' (SEQ ID NO:28);
5' - CGG TAG TAT TGC CC TTA AGC C - 3' (SEQ ID NO:29); and full-length
complements thereof.
10. (AMENDED) A primer selected from the group of:
5' - CGG AAA AGC ACG TCG ATG GG - 3' (SEQ ID NO:30);
5' - GCG ATA TCG TTG GTG GTG CC - 3' (SEQ ID NO:31); and full-length
complements thereof.

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11. (AMENDED) A primer selected from the group of:

5' - CTC GAT GAT GCG TGC TTC GC - 3' (SEQ ID NO:32);

5' - GCG ACT GTG ATG TAT AAA CG - 3' (SEQ ID NO:33); and **full-length**

complements thereof.

17. A method for identifying a beta-lactamase in a clinical sample, the method comprising:

providing a pair of oligonucleotide primers, wherein one primer of the pair is

complementary to at least a portion of the beta-lactamase nucleic acid in the sense strand and the other primer of each pair is complementary to at least a portion of the beta-lactamase nucleic acid in the antisense strand;

annealing the primers to the beta-lactamase nucleic acid;

simultaneously extending the annealed primers from a 3' terminus of each primer to synthesize an extension product that is complementary to the nucleic acid strands annealed to each primer wherein each extension product after separation from the beta-lactamase nucleic acid serves as a template for the synthesis of an extension product for the other primer of each pair;

separating the amplified products; and

analyzing the separated amplified products for a region characteristic of the beta-lactamase.

18. The method of claim 17 wherein the primers are specific for nucleic acid characteristic of the TEM family of beta-lactamase enzymes.

19. (AMENDED) The method of claim 18 wherein the primers are selected from the group of:

5' - CTT GGT CTG ACA GTT ACC - 3' (SEQ ID NO:3);

5' - TGT CGC CCT TAT TCC - 3' (SEQ ID NO:4);

5' - TCG GGG AAA TGT GCG - 3' (SEQ ID NO:5); and **full-length** complements

thereof.

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20. The method of claim 17 wherein the primers are specific for nucleic acid characteristic of the SHV family of beta-lactamase enzymes.

21. (AMENDED) The method of claim 20 wherein the primers are selected from the group of:

5' - ATC GTC CAC CAT CCA CTG CA - 3' (SEQ ID NO:6);

5' - GGG AAA CGG AAC TGA ATG AG - 3' (SEQ ID NO:7);

5' - TAG TGG ATC TTT CGC TCC AG - 3' (SEQ ID NO:8);

5' - GCT CTG CTT TGT TAT TC - 3' (SEQ ID NO:9);

5' - CAC TCA AGG ATG TAT TGT G - 3' (SEQ ID NO:10); and **full-length**

complements thereof.

24. The method of claim 17 wherein the primers are specific for nucleic acid characteristic of the AmpC beta-lactamase enzyme found in *Citrobacter freundii*.

25. (AMENDED) The method of claim 24 wherein the primers are selected from the group of:

5' - CTG GCA ACC ACA ATG GAC TCC G - 3' (SEQ ID NO:18);

5' - GCC AGT TCA GCA TCT CCC AGC C - 3' (SEQ ID NO:19); and **full-length**

complements thereof.

26. The method of claim 17 wherein the primers are specific for nucleic acid characteristic of the AmpC beta-lactamase enzyme found in *Serratia marcescens*.

27. (AMENDED) The method of claim 26 wherein the primers are selected from the group of:

5' - CGT GAC CAA CAA CGC CCA GC - 3' (SEQ ID NO:20);

5' - CCA GAT AGC GAA TCA GAT CGC - 3' (SEQ ID NO:21); and **full-length**

complements thereof.

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30. The method of claim 17 wherein the primers are specific for nucleic acid characteristic of the AmpC beta-lactamase enzyme found in *Pseudomonas aeruginosa*.

31. (AMENDED) The method of claim 30 wherein the primers are selected from the group of:

5' - GGC ATT GGG ATA GTT GCG GTT G - 3' (SEQ ID NO:24);

5' - TTA CTA CAA GGT CGG CGA CAT GAC C - 3' (SEQ ID NO:25); and **full-length** complements thereof.

32. The method of claim 17 wherein the primers are specific for nucleic acid characteristic of the AmpC beta-lactamase enzyme found in *E. coli*.

33. (AMENDED) The method of claim 32 wherein the primers are selected from the group of:

5' - GGA TCA CAC TAT TAC ATC TCG C - 3' (SEQ ID NO:26);

5' - CGT ATG GTT GAG TTT GAG TGG C - 3' (SEQ ID NO:27); and **full-length** complements thereof.

34. The method of claim 17 wherein the primers are specific for nucleic acid characteristic of the K1 beta-lactamase enzyme.

35. (AMENDED) The method of claim 34 wherein the primers are selected from the group of:

5' - GCG ACC TGG TTA ACT ACA ATC CC - 3' (SEQ ID NO:28);

5' - CGG TAG TAT TGC CC TTA AGC C - 3' (SEQ ID NO:29); and **full-length** complements thereof.

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36. (AMENDED) The method of claim 34 wherein the primers are selected from the group of:

5' - CGG AAA AGC ACG TCG ATG GG - 3' (SEQ ID NO:30);

5' - GCG ATA TCG TTG GTG GTG CC - 3' (SEQ ID NO:31); and **full-length** complements thereof.

37. The method of claim 17 wherein the primers are specific for nucleic acid characteristic of the PSE1, PSE4, and CARB3 beta-lactamase enzymes.

38. (AMENDED) The method of claim 37 wherein the primers are selected from the group of:

5' - CTC GAT GAT GCG TGC TTC GC - 3' (SEQ ID NO:32);

5' - GCG ACT GTG ATG TAT AAA CG - 3' (SEQ ID NO:33); and **full-length** complements thereof.

49. (AMENDED) A diagnostic kit for detecting a TEM family beta-lactamase which comprises packaging, containing, separately packaged:

(a) at least one primer pair capable of hybridizing to a beta-lactamase nucleic acid of interest wherein at least one of the primers is selected from the primers of claim 1;

(b) a positive and negative control; and

(c) a protocol for identification of the beta-lactamase nucleic acid of interest.

50. (AMENDED) The diagnostic kit of claim 49 wherein the primers are selected from the group of:

5' - CTT GGT CTG ACA GTT ACC - 3' (SEQ ID NO:3);

5' - TGT CGC CCT TAT TCC - 3' (SEQ ID NO:4);

5' - TCG GGG AAA TGT GCG - 3' (SEQ ID NO:5); and **full-length** complements thereof.

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51. (AMENDED) A diagnostic kit for detecting a SHV family beta-lactamase which comprises packaging, containing, separately packaged:

- (a) at least one primer pair capable of hybridizing to a beta-lactamase nucleic acid of interest wherein at least one of the primers is selected from the primers of claim 2;
- (b) a positive and negative control; and
- (c) a protocol for identification of the beta-lactamase nucleic acid of interest.

52. (AMENDED) A diagnostic kit for detecting an AmpC family beta-lactamase which comprises packaging, containing, separately packaged:

- (a) at least one primer pair capable of hybridizing to a beta-lactamase nucleic acid of interest;
- (b) a positive and negative control; and
- (c) a protocol for identification of the beta-lactamase nucleic acid of interest.

53. (AMENDED) The kit of claim 52 wherein at least one of the primers is selected from the group consisting of:

- 5' - CTG GCA ACC ACA ATG GAC TCC G - 3' (SEQ ID NO:18);
- 5' - GCC AGT TCA GCA TCT CCC AGC C - 3' (SEQ ID NO:19);
- 5' - CGT GAC CAA CAA CGC CCA GC - 3' (SEQ ID NO:20);
- 5' - CCA GAT AGC GAA TCA GAT CGC - 3' (SEQ ID NO:21);
- 5' - GGC ATT GGG ATA GTT GCG GTT G - 3' (SEQ ID NO:24);
- 5' - TTA CTA CAA GGT CGG CGA CAT GAC C - 3' (SEQ ID NO:25);
- 5' - GGA TCA CAC TAT TAC ATC TCG C - 3' (SEQ ID NO:26);
- 5' - CGT ATG GTT GAG TTT GAG TGG C - 3' (SEQ ID NO:27); and full-length

complements thereof.

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54. (AMENDED) A diagnostic kit for detecting a K1 family beta-lactamase which comprises packaging, containing, separately packaged:

(a) at least one primer pair capable of hybridizing to a beta-lactamase nucleic acid of interest;

(b) a positive and negative control; and

(c) a protocol for identification of the beta-lactamase nucleic acid of interest.

55. (AMENDED) The kit of claim 54 wherein at least one of the primers is selected from the group consisting of:

5' - GCG ACC TGG TTA ACT ACA ATC CC - 3' (SEQ ID NO:28);

5' - CGG TAG TAT TGC CC TTA AGC C - 3' (SEQ ID NO:29);

5' - CGG AAA AGC ACG TCG ATG GG - 3' (SEQ ID NO:30);

5' - GCG ATA TCG TTG GTG GTG CC - 3' (SEQ ID NO:31); and **full-length** complements thereof.

56. (AMENDED) A diagnostic kit for detecting a PSE1, PSE4, or CARB3 family beta-lactamase which comprises packaging, containing, separately packaged:

(a) at least one primer pair capable of hybridizing to a beta-lactamase nucleic acid **characteristic of [interest] the PSE1, PSE4, or CARB3 families of beta-lactamase enzymes;**

(b) a positive and negative control; and

(c) a protocol for identification of the beta-lactamase nucleic acid **characteristic of [interest] the PSE1, PSE4, or CARB3 families of beta-lactamase enzymes.**

57. (AMENDED) The kit of claim 56 wherein at least one of the primers is selected from the group consisting of:

5' - CTC GAT GAT GCG TGC TTC GC - 3' (SEQ ID NO:32);

5' - GCG ACT GTG ATG TAT AAA CG - 3' (SEQ ID NO:33); and **full-length** complements thereof.